OSTEOPROTEGERIN, RANK, RANKL

David Stejskal^a, Josef Bartek^b, Radmila Pastorková^a, Viktor Růžička^a,
Ivo Oral^a, Dalimil Horalík^a

^a Department of Laboratory Medicine and Internal Medicine, Hospital Šternberk

^b Institute of Medical Chemistry and Biochemistry, Palacký University, 775 15 Olomouc

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Osteoprotegerin, RANK (Receptor Activator of Nuclear factor kappa B) and RANKL (Receptor Activator of Nuclear factor kappa B ligand) became the aim of intensive research. RANK is considered as a hematopoietic surface receptor controlling osteoclastogenesis and calcium metabolism. RANKL may promote osteoresorption by induction of cathepsin K gene expression. The present paper summarizes the most significant data in osteoprotegerin, RANK and RANKL problems obtained.

INTRODUCTION

In the membrane of cells of osteoclast line and in dendritic cells, there is a protein crucial for all calcium-tropic hormones and proresorptive cytokines to increase calcemia and multiplication of osteoclasts in the bone. This protein was identified as RANK (Receptor Activator of Nuclear factor Kappa B)¹⁰. RANK is considered as a hematopoietic surface receptor controlling osteoclastogenesis⁶ and calcium metabolism. RANK ligand and antagonist is a protein produced by osteoblasts, cells of bone stroma and by activated T lymphocytes. It was identified as RANKL (RANK ligand); it may promote osteoresorption by induction of cathepsin K gene expression^{10, 30}. Osteoblasts and stromal cells produce another protein, which binds RANKL and interferes with its bond on RANK. This protein inhibits differentiation of progenitors into osteoblasts, displays hypocalcemic and antiresorptive effects. It was named osteoprotegerin (OPG) or OCIF (Osteoclastogenesis Inhibiting Factor)^{1, 10}. RANKL and its inhibitor osteoprotegerin are decisive for differentiation and osteoresorption function of the osteoclast so that they became the aim of intensive research. Both the molecules occur in a free form, not bound on a membrane, their concentration can be measured and probably applied in diagnostics.

Recently, several communications, experimental and clinical studies dealt with OPG and RANKL. However, contradictory conclusions were drawn from these studies. Therefore the present paper summarizes the most significant data obtained.

OSTEOPROTEGERIN, RANK, RANKL*Pathophysiological mechanisms of OPG/RANKL effects*

Osteoprotegerin (OPG) and RANKL are cytokines regulating osteoclastogenesis. Both agents are classified

into the superfamily of TNF and TNF receptor. RANKL binds on receptors on the surface of preosteoclasts and stimulates their differentiation into active osteoclasts, thus resulting in osteoresorption. It probably acts synergically with TNF-alpha (TNF acts via TNF-1 receptor and leads to a massive osteoclastogenesis after RANKL effect)³. OPG inhibits this osteoclastogenesis. It is a receptor for RANKL, which is produced by osteoblasts and binds on RANKL, thus inhibiting maturation of osteoclasts. This may lead even to osteopetrosis, which is caused by and associated with a small amount of osteoclasts and their functional inability¹. OPG and RANKL are supposed to have many other functions in the organism (influence immune processes, origin of vascular calcifications and calcium metabolism in general, metabolism of mammary gland during pregnancy, etc.)²⁸. The mRNA OPG is detectable in bones and also in soft tissues – digestive tract, heart, lungs, liver, kidney, brain, maybe in cartilage; on the contrary, RANKL expression is limited to areas of osteoresorption and lymph nodes.

Control of the OPG/RANKL system in the organism

Concentration of OPG and RANKL (osteoclast differentiation) is controlled by many osteotropic hormones and cytokines. Agents reducing OPG/RANKL ratio: Glucocorticoids (enhance osteoclastogenesis by OPG inhibition and OPGL production via cells of osteoblastic line, which leads to increased osteoresorption), some inflammatory cytokines (IL-1 beta, IL-4, IL-6, IL-11, IL-17, TNF-alpha, they stimulate osteoclastogenesis by induction of OPGL expression), basic fibroblast growth factor 2 (inhibits OPG production and stimulates RANKL production), PTH (inhibits OPG production and promotes RANKL production), prostaglandins E2, various mesenchymal transcription factors (cbfa-1, PPAR-gamma), 1.25 OH vitamin D^{3, 4, 8, 10, 14, 27, 28}.

Agents enhancing OPG/RANKL ratio: Estrogens (enhance OPG secretion by osteoblastic cells and inhibit RANKL production – this effect is supposed to play an important role in the anti-resorption effect of estrogens on the bone), TGF-beta (induces OPG secretion)^{2, 5, 29}.

Verification of OPG/RANKL effects in the organism under experimental conditions

Several months ago, the paper was published on transgenic mice line from which was removed the functional gene coding RANK (RANK^{-/-}); knock-out of a gene for RANK resulted in the following changes: absence of osteoclasts, enchondral ossification was impaired, mice had a severe osteopetrosis¹². Contrary to that, another experiment proved that OPG-deficient mice had osteoporosis (RANKL is not regulated, graded osteoresorption occurs) and show an increased presence of premature arterial calcifications (this supports the hypothesis on OPG/RANKL effect on regulation of vascular calcification)^{2, 9, 12, 26}.

Clinical studies of OPG/RANKL

To present, sporadic clinical studies were performed and the results obtained are contradictory. These discrepancies may be due to different methods of selection of probands for the study or the pre-analytic phase of OPG determination (sample storage). In addition, OPG/RANKL ratio is decisive for clinical interpretation; however, a commercial set for determination of RANKL concentration is not available. Parameters of bone remodeling showed certain associations with OPG only sporadically; e. g. OPG and osteocalcine, deoxypyridinoline and OPG, and OPG and C-terminal collagen I propeptide^{2, 22}; on the other hand, other studies did not prove any correlations between bone markers and OPG³¹. Some authors proved slight negative correlations between OPG and calcium concentration and slight positive correlations between OPG and PTH³. It appears that OPG concentration in human correlates with age (values increase with ageing); however, a slight correlation with concentration of free testosterone and estradiol was reported. It is probable that in the above-mentioned cases the OPG concentrations are increased, but OPG/RANKL ratio is decreased and increased, respectively^{2, 31}. Only few human clinical studies proved correlation between OPG and bone density but other studies did not find any correlation^{2, 18, 31}. No unambiguous associations were proved between OPG concentration and occurrence of osteoporotic fractures or risk of their origin (only one ad hoc performed analysis of several women with collum fractures reported a possible correlation between OPG and risk of their occurrence)². Nevertheless, the pathogenetic model OPG/RANKL has been applied in studies on malignant primary and secondary bone lesions; a lesion appears to have increasing

levels of both OPG and RANKL but decreasing OPG/RANKL ratio^{4, 12}. Some authors confirmed that OPG increases in patients with metastatic spread of the skeleton in prostate carcinoma (OPG increases both in systemic circulation and metastatic bone focus)^{15, 17}. Patients with the locally graded osteolysis after implantation of prosthesis also have in the area of osteolytic lesions a high expression of RANK and RANKL. This may be due to stimulation of macrophages by particles of prosthetic materials¹¹. A decreased OPG concentration and reduced OPG/RANKL ratio also occur in patients with myeloma; OPG concentrations correlate negatively with the grade of skeleton destruction (application of OPG could be favorable in this case)^{22, 24}. The system OPG/RANKL has been studied also in rheumatoid diseases because they are often associated with changes of bone metabolism (osteoresorption intensifies due to excessive production of cytokines and other growth factors). It was found that patients with rheumatoid arthritis had elevated OPG concentration in the synovial fluid; such elevation is probably secondary (as reaction of the organism to intensified osteoresorption) and probands may have a reduced ratio of OPG/RANKL^{14, 19}. With regard to the supposed pathophysiological effects of OPG, association was studied between the system OPG/RANKL and pathophysiology of vascular calcification. It was found that incidence of arterial calcification is related to balance of the system OPG/RANKL and that administration of OPG could probably inhibit the occurrence of arterial calcifications (e. g. caused experimentally by administration of warfarin or vitamin D). Moreover, a higher level of OPG was proved to correlate with higher cardiovascular (4.4×) and total mortality (3×); a decisive limit was >150 ng/l). Correlations between OPG and other risk factors (BMI, HDL, LDL) have not been found yet. No association was found between OPG and the incidence of thrombotic strokes². The same authors proved that OPG concentration in diabetic women correlated negatively with fructosamine concentration; OPG is supposed to increase at impaired compensation of diabetes mellitus. One hypothesis is based on the fact that the concentration of glycated proteins interferes with OPG measurement. Another hypothesis suggests a correlation between OPG and vascular calcification, which occurs more frequently in diabetic patients and leads to cardiovascular complications. Another possible cause of enhanced OPG level could be the OPG binding on RANKL and subsequently a reduced OPG clearance². The experimental animal models showed that OPG concentration enhanced considerably during pregnancy (a protective role could be supposed in the prevention of osteoporosis in pregnant women)¹⁸. OPG increases in patients with severe renal failure; preliminary results indicate that this is due to OPG clearance (immediately after renal transplantation the OPG levels decrease)²⁰. In patients with renal osteodystrophy the OPG levels are increased but the OPG/RANKL index is reduced²¹.

Utilization of the OPG/RANKL system in therapy

With regard to a supposed favorable effect of OPG administration to individuals with osteoporosis, first animal and then human studies were performed with application of OPG to individuals with severe osteoporosis or destructive diseases of the skeleton. Osteoprotegerin was produced by recombinant method and administered experimentally in order to reduce or stop osteoresorption and bone invasion in metastasizing mammary, colon and prostate carcinomas, osteosarcoma, after ovariectomy and in experimental osteoporosis. Most intervention studies proved a significant effect of osteoprotegerin (minimized development of experimental bone metastases, reduced development of osteoporosis caused by ovariectomy or motor inactivity)⁹. The animal experiment proved that the administration of OPG resulted in a significant reduction of the experimentally induced vascular calcifications (application of vitamin D or warfarin). This supported the hypothesis on correlation between the origin of vascular calcifications and certain skeleton diseases^{16, 25}. The application of OPG also resulted in decreased hypercalcemia and a significant and long-term reduction of osteoresorption was directly associated with the administration of HRT^{2, 7, 8}. With respect to the aforementioned results, the application of OPG has been discussed for palliative therapy of metastasizing tumors, severe, mostly secondary osteoporoses (myeloma, rheumatoid arthritis, administration of corticoids, immobilization, renal failure etc.), and for patients with Paget disease or periodontal diseases^{9, 10, 12, 23, 24, 32}.

CONCLUSION

The OPG/RANKL system determines significantly osteoclastogenesis and osteoresorption. Results obtained from experimental and clinical studies indicate that this system could be applied in diagnostics and therapy (assessment of OPG/RANKL in risk individuals, monitoring of therapy efficiency at defined concentration of OPG/RANKL, administration of OPG to threatened groups of probands).

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